HELLER EHRMAN WHITE & MCAULIFFE ELP
Sheet 1 of 12

Title: HIGH THROUGHPUT DIRECTED EVOLUTION BY
RATIONAL MUTAGENESIS

Applicant: VEGA et al.
Filed: December 17, 2001 Appl. No.: 10/022,249

Examiner: Unassigned. Art Unit: 1643

Our Docket No.: 37851-0911 complete library testing, clone-by-clone identification of HITS 00000000 Phenotypic charact. 1st Round: screening of mutants (full length Ala-scan) 00000000 00000000 production HT virus 1st mutant library (Ala-scan) (gene)

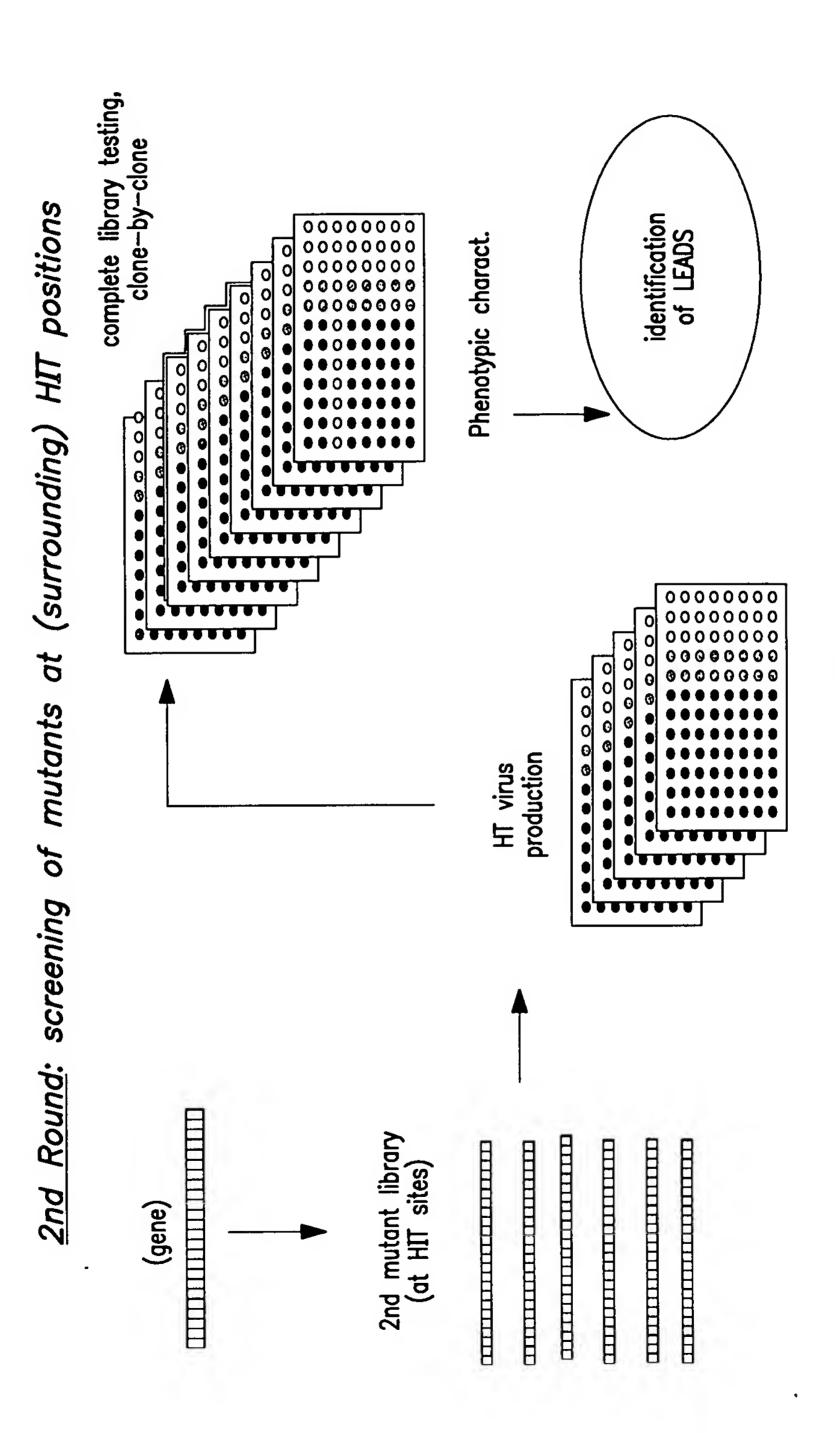
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Title: HIGH THROUGHPUT DIRECTED EVOLUTION BY
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Examinary Unassigned Art Unit: 1643

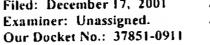
Examiner: Unassigned. Our Docket No.: 37851-0911

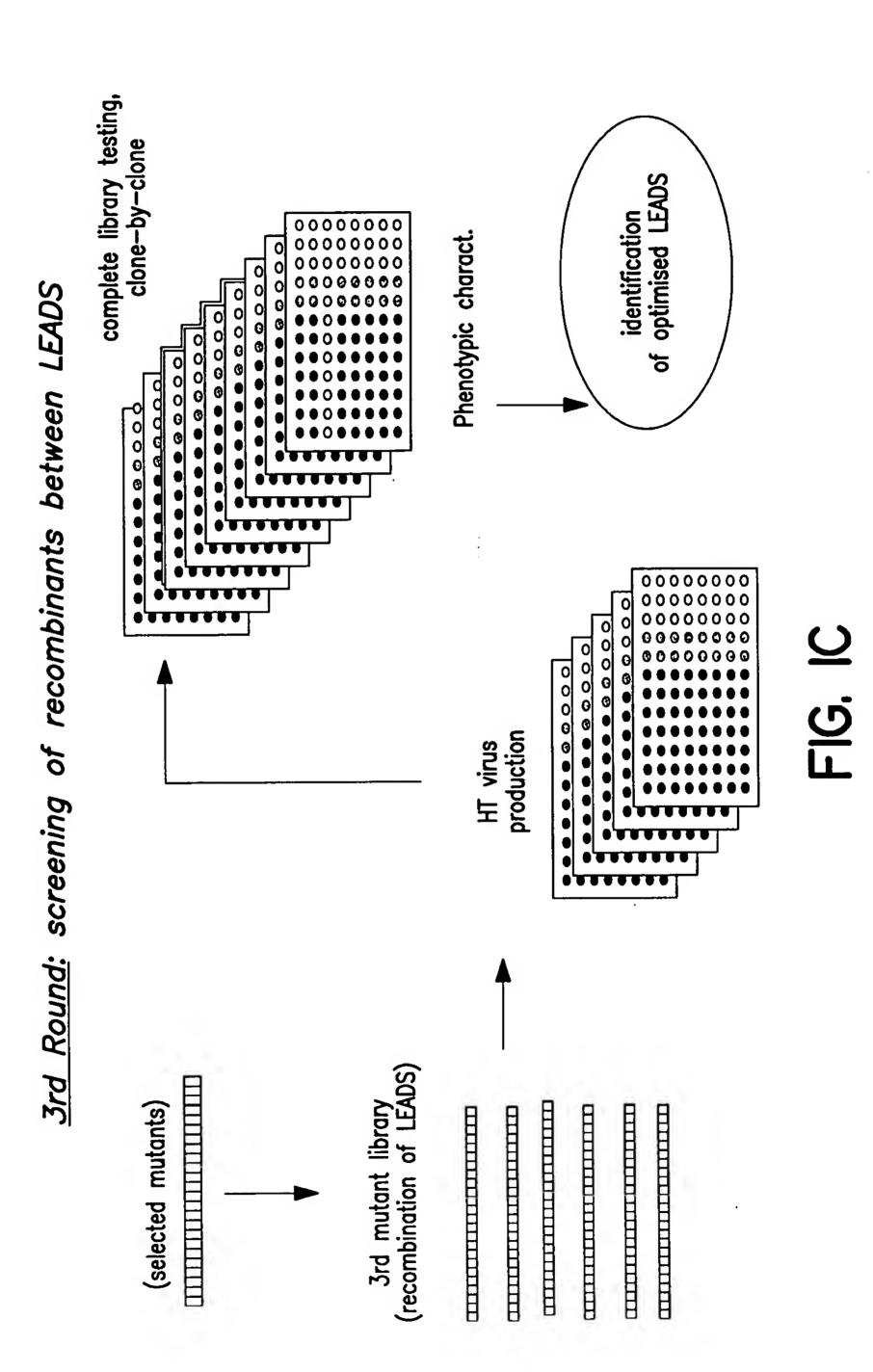


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Title: HIGH THROUGHPUT DIRECTED EVOLUTION BY
RATIONAL MUTAGENESIS
Applicant: VEGA et al.
Filed: December 17, 2001 Appl. No.: 10/022,249

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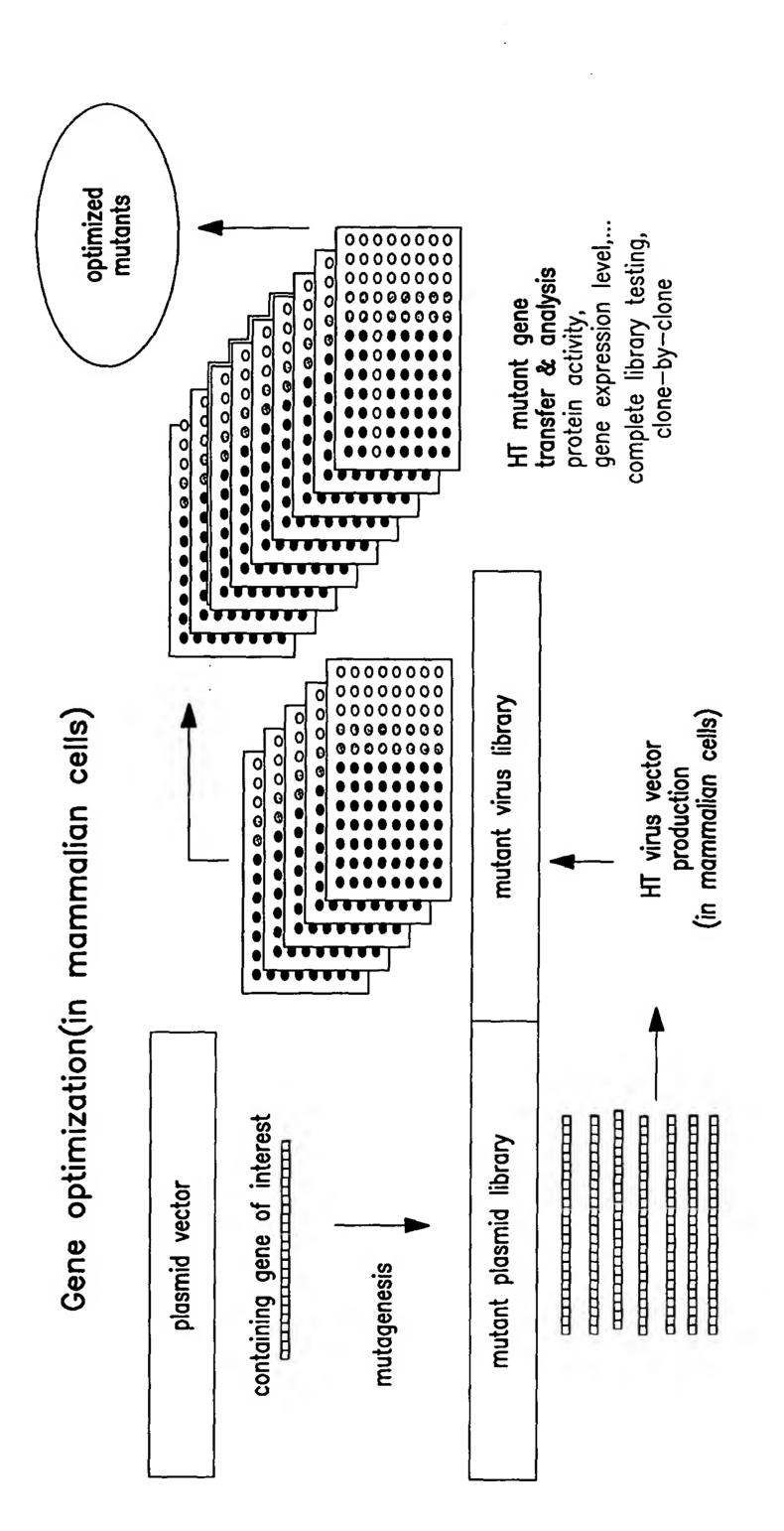
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Title: HIGH THROUGHPUT DIRECTED EVOLUTION BY
RATIONAL MUTAGENESIS

Applicant: VEGA et al.
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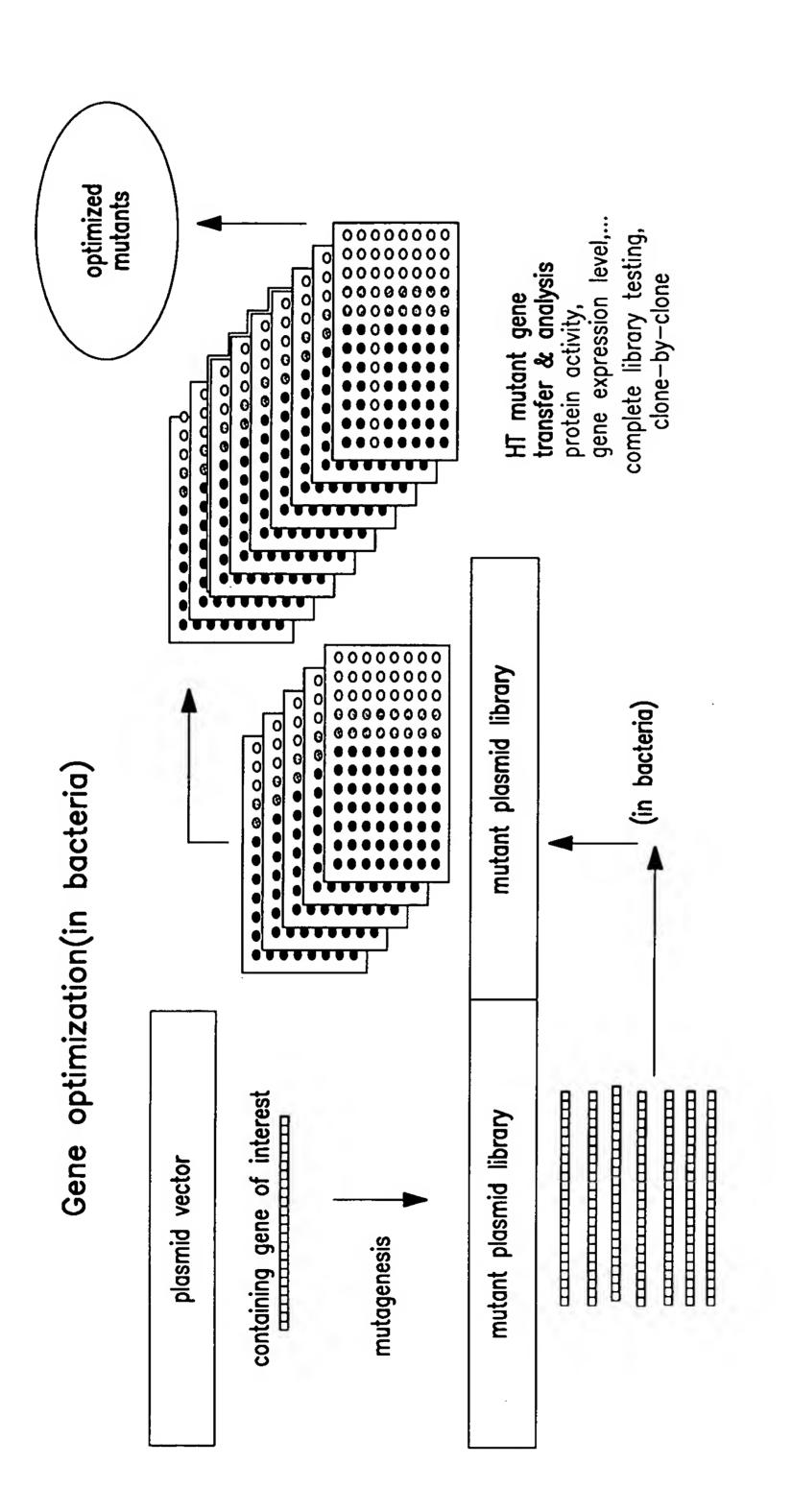
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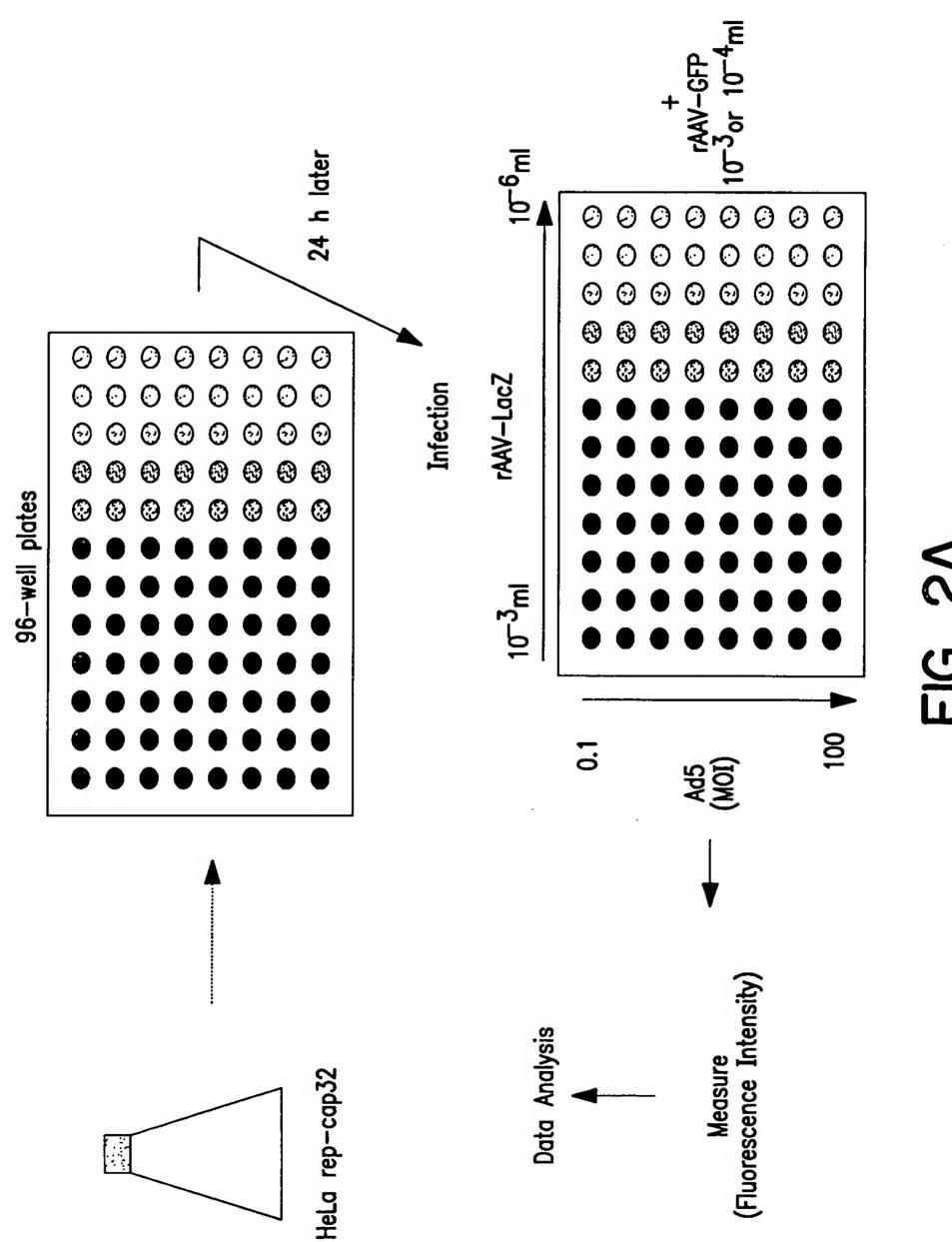
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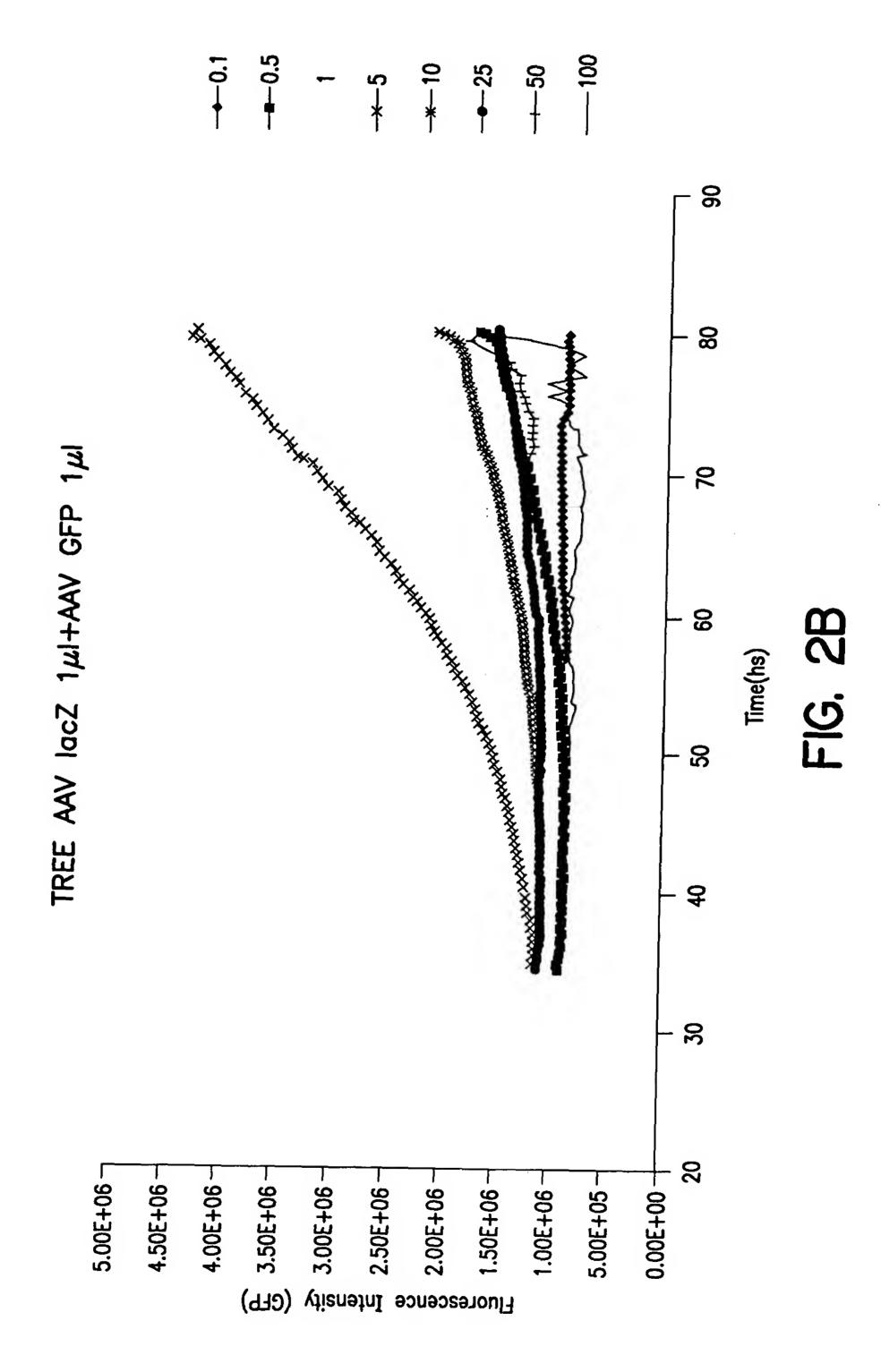
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Applicant: VEGA et al.
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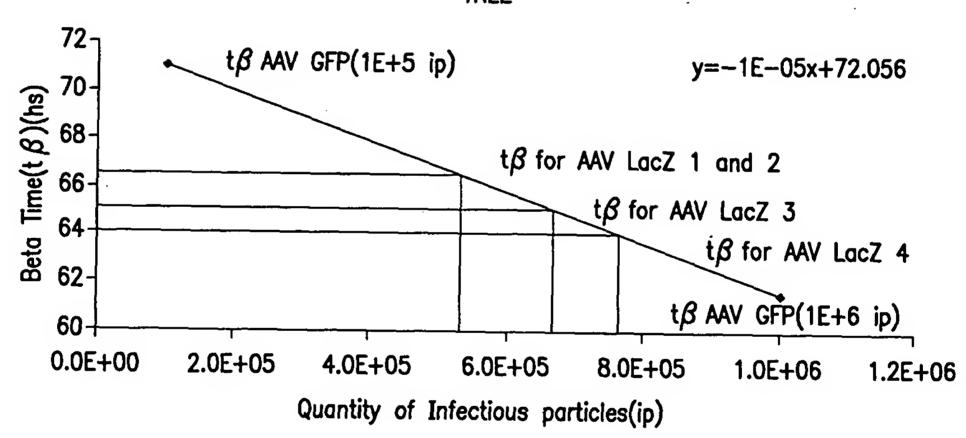


FIG. 2C

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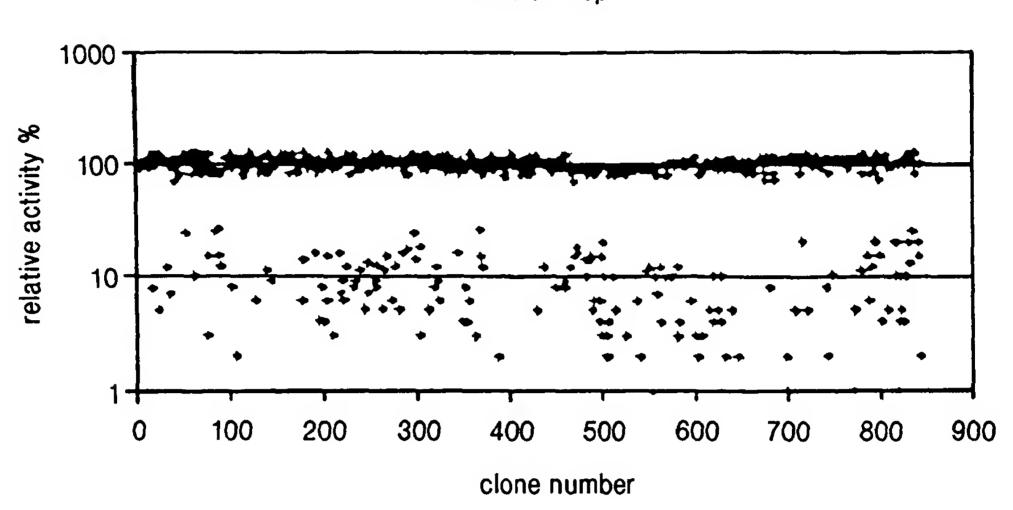


FIG. 3A

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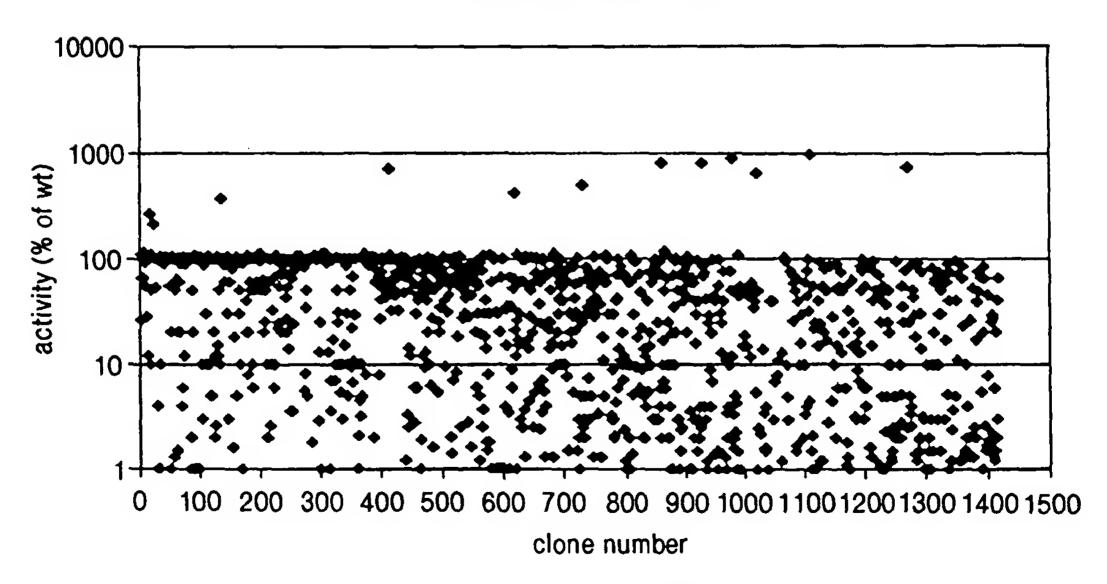


FIG. 3B

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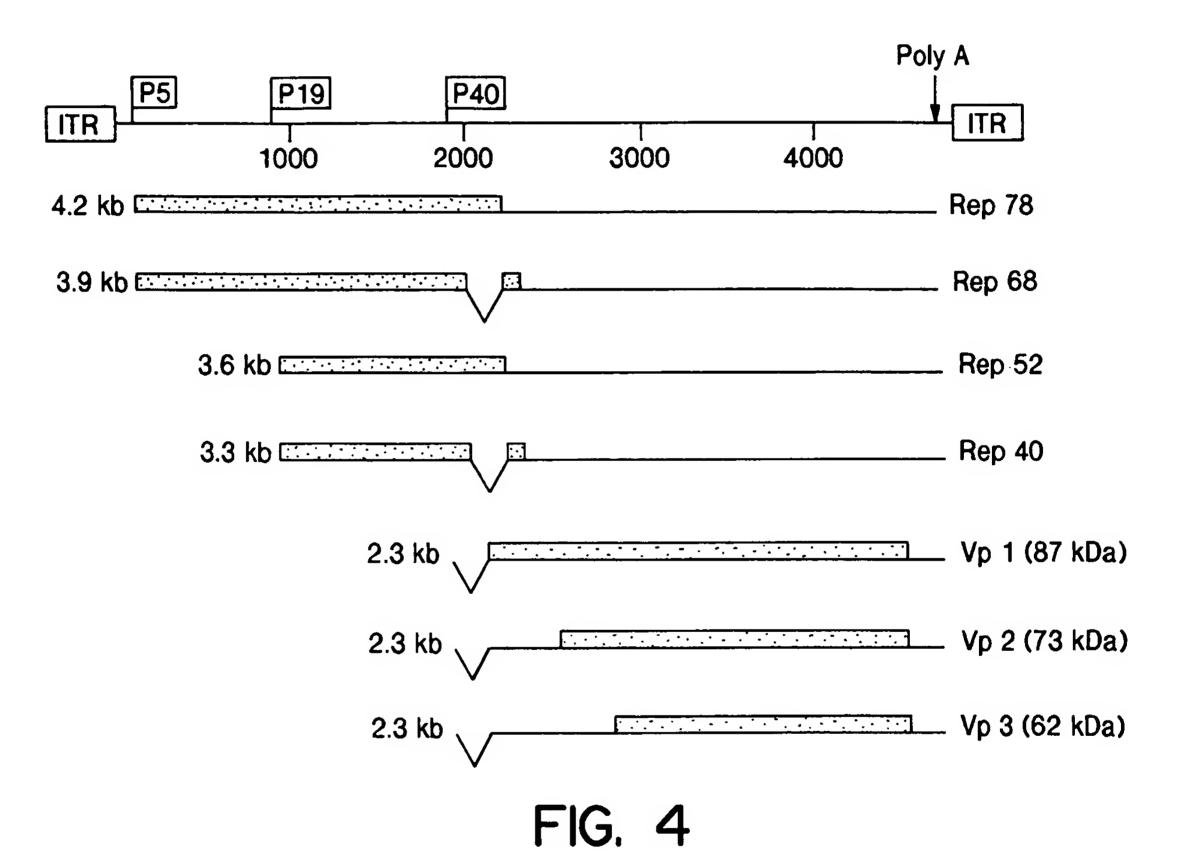
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4			60
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_	70 80 90 100 110	120	
1	RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHILVETTGVKSMVLGRFLSQIRDK	_	120
2	RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHILVETTGVKSMVLGRFLSQIRDK		120
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4	REFLVEWRRVSKAPEALFFVQFEKGETYFHLHVLIETIGVKSMVVGRYVSQIKEK	LVTRI	120
5	REFLVEWRRVSKAPEALFFVQFEKGDSYFHLHILVETVGVKSMVVGRYVSQIKEK	LVTRI	120
6	RDFLTEWRRVSKAPEALFFVQFEKGESYFHMHVLVETTGVKSMVLGRFLSQIREK	LIQRI	120
7	RVFLYEWNKFSKQ-ESKFFVQFEKGSEYFHLHTLVETSGISSMVLGRYVSQIRAQ	LVKVV	119
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2 3 4 5	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISS	300 NRIYR NRIYR NRIYQ NRIYQ NRIYR	300 300 300 300
2 3 4 5	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISS QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISS	300 NRIYR NRIYR NRIYQ NRIYQ NRIYR NRIYR	300 300 300 300 300
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2 3 4 5 6 7 C	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQQPVEDISS QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISS QWIQEDQASYISFNAASNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQENQESYLSFNSTGNSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI:: 310 320 330 340 350 ILELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG	300 NRIYR NRIYR NRIYQ NRIYQ NRIYQ NRIYR NRIYK NRIYK NRIWQ NRI*:	300 300 300 300 300 296
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234567C 12345	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISS QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISS QWIQENQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI:: 310 320 330 340 350 ILELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG	300 NRIYR NRIYR NRIYQ NRIYQ NRIYR NRIYK NRIWQ NRI*: 360 CVNWT CVNWT CVNWT	300 300 300 300 296 360 360 360 360
234567C 123456	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISS QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISS QWIQEDQASYISFNAASNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQENQESYLSFNSTGNSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI:: 310 320 330 340 350 ILELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG	300 NRIYR NRIYR NRIYQ NRIYQ NRIYR NRIYK NRIWQ NRI*: 360 CVNWT CVNWT CVNWT	300 300 300 300 296 360 360 360 360 360
234567C 1234567	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGGNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISS QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISS QWIQEDQASYISFNAASNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQEAQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI:: 310 320 330 340 350 ILELNGYPPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPAYAGSVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILENGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWCQRSFNKRNTVWLYGPATTGKTNIAEAIAHTVPFYG	300 NRIYR NRIYR NRIYQ NRIYQ NRIYR NRIYK NRIWQ NRI*: 360 CVNWT CVNWT CVNWT CVNWT	300 300 300 300 296 360 360 360 360
234567C 123456	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTKSAPDYLVGPAPPADIKT QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITK QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEDISS QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQQPVEDISS QWIQEDQASYISFNAASNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQENQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISK QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI:: 310 320 330 340 350 ILELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPAYAGSVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIAHAVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG ILELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIAHTVPFYG	300 NRIYR NRIYR NRIYQ NRIYQ NRIYR NRIYK NRIWQ NRI*: 360 CVNWT CVNWT CVNWT CVNWT	300 300 300 300 296 360 360 360 360 360

HELLER EHRMAN WHITE & MCAULIFFE LLP Sheet 12 of 12 Title: HIGH THROUGHPUT DIRECTED EVOLUTION BY RATIONAL MUTAGENESIS Applicant: VEGA et al. Filed: December 17, 2001 Appl. No.: 10/022,249 Examiner: Unassigned. Art Unit: 1643 Our Docket No.: 37851-0911

	370 380 390 400 410 420	4.0.0
1	NENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTS	420
2	NENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTS	420
3	NENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIEPTPVIVTS	420
4	NENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIEPTPVIVTS	420
5	NENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTS	420
6	NENFPFNDCVDKMVIWWEEGKMTAKVVESAKAILGGSKVRVDQKCKSSAQIDPTPVIVTS	420
7	NENFPFNDCVDKMLIWWEEGKMTNKVVESAKAILGGSKVRVDQKCKSSVQIDSTPVIVTS	416
С	NENFPFNDCVDKM*IWWEEGKMT*KVVESAKAILGGSKVRVDQKCKSS*QI+*TPVIVTS	
	430 440 450 460 470 480	
1	NTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLEHDFGKVTKQEVKEFFRWAQDHVTEV	480
2	NTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLEHDFGKVTKQEVKEFFRWAQDHVTEV	480
3	NTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWASDHVTDV	480
4	NTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWASDHVTDV	480
5	NTNMCAVIDGNSTTFEHQQPLQDRMFKFELTKRLEHDFGKVTKQEVKDFFRWASDHVTEV	480
6	NTNMCAVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDFGKVTKQEVKDFFRWAKDHVVEV	480
7	NTNMCVVVDGNSTTFEHQQPLEDRMFKFELTKRLPPDFGKITKQEVKDFFAWAKVNQVPV	476
Ċ	NTNMC*V*DGNSTTFEHQQPL*DRMFKFELT+RL:*DFGK*TKQEVK+FF*WA:***+:V	
	490 500 510 520	
1	AHEFYVRKGGANKRPAPDDADKSEPKRA	522
2	AHEFYVRKGGANKRPAPDDADKSEPKRA	522
3	AHEFYVRKGGAKKRPASNDADVSEPKRQCTSLAQPTTSDAEA	522
ے 4	AHEFYVRKGGAKKRPASNDADVSEPKRQCTSLAQPTTSDAEA	522
5	THEFYVRKGGARKRPAPNDADISEPKRA	522
	EHEFYVKKGGAKKRPAPSDADISEPKRV	522
6		536
7	THEFKVPRELAGTKGAEKSLKRPLGDVTNTSYKSLEKRARLSFVPETPRSSDVTVDPAPL:HEF*V+**A:***A::***************************	220
С	:HEF*V+**A:***A::***A::**A::	
	530 540 550 560 570 580	
_		580
1	APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFPGVSESQ	
2	APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFPGVSESQ	580
3	P-ADYADRYQNKCSRHVGMNLMLFPCKTCERMNQISNVCFTHGQRDCGECFPGMSESQPV	581
4	P-ADYADRYQNKCSRHVGMNLMLFPCKTCERMNQISNVCFTHGQRDCGECFPGMSESQPV	581
5	P-VDYADRYQNKCSRHVGMNLMLFPCRQCERMNQNVDICFTHGVMDCAECFP-VSESQPV	580
6	S-INYADRYQNKCSRHVGMNLMLFPCRQCERMNQNSNICFTHGQKDCLECFPVSESQP	579
7	RPLNWNSRYDCKCDYHAQFDNISNKCDECEYLNRGKNGCICHNVTHCQICHG	588
C	:::+:**RY**KC**H:**::****C::CE**N*::*:C**H*::*C.*C**::+:::	
	590 600 610 620	
1	PVVRKRTYRKLCAIHHLLGRAPEIACSACDLVNVDLDDCVSEQ	623
2	PVVRKRTYRKLCAIHHLLGRAPEIACSACDLVNVDLDDCVSEQ	623
3	SVVKKKTYQKLCPIHHILGRAPEIACSACDLANVDLDDCVSEQ	624
4	SVVKKKTYQKLCPIHHILGRAPEIACSACDLANVDLDDCVSEQ	624
5	SVVRKRTYQKLCPIHHIMGRAPEVACSACELANVDLDDCDMEQ	623
6	VSVVKKAYQKLCYIHHIMG-KVPDACTACDLVNVDLDDCIFEQ	621
7		610
C	:+*:*:+*:***:***::*++++:**+**+*:**D*DD*::EQ	

FIG. 5B